Equine Botulinum Antitoxin for the Treatment of Infant Botulism[∇]

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**Infant botulism is the most common form of human botulism in Argentina and the United States. BabyBIG
(botulism immune globulin intravenous [human]) is the antitoxin of choice for specific treatment of infant
botulism in the United States. However, its high cost limits its use in many countries. We report here the
effectiveness and safety of equine botulinum antitoxin (EqBA) as an alternative treatment. We conducted an
analytical, observational, retrospective, and longitudinal study on cases of infant botulism registered in
Mendoza, Argentina, from 1993 to 2007. We analyzed 92 medical records of laboratory-confirmed cases and
evaluated the safety and efficacy of treatment with EqBA. Forty-nine laboratory-confirmed cases of infant
botulism demanding admission in intensive care units and mechanical ventilation included 31 treated with
EqBA within the 5 days after the onset of signs and 18 untreated with EqBA. EqBA-treated patients had a
reduction in the mean length of hospital stay of 23.9 days ($P = 0.0007$). For infants treated with EqBA, the
intensive care unit stay was shortened by 11.2 days ($P = 0.0036$), mechanical ventilation was reduced by
11.1 days ($P = 0.0155$), and tube feeding was reduced by 24.4 days ($P = 0.0001$). The incidence of sepsis
in EqBA-treated patients was 47.3% lower ($P = 0.0017$) than in the untreated ones. Neither sequelae nor
adverse effects attributable to EqBA were noticed, except for one infant who developed a transient
erythematous rash. These results suggest that prompt treatment of infant botulism with EqBA is safe and
effective and that EqBA could be considered an alternative specific treatment for infant botulism when
BabyBIG is not available.**

Infant botulism is an intestinal toxemia that affects infants younger than 1 year, and it occurs usually between 2 and 24 weeks of age (3). Infant botulism should be strongly suspected in any infant who presents constipation (more than 3 days without defecation), sluggish or fixed pupils, and any sign of muscle hypotonia (20, 21). Infant botulism occurs when swallowed botulinum spores germinate, and then vegetative cells multiply, temporally colonize the large intestine, and synthesize botulinum neurotoxin (BoNT) *in situ*. The neurotoxin is absorbed and carried out by the bloodstream to neuromuscular junctions, where it blocks the release of acetylcholine, causing a flaccid paralysis. Infant botulism presents a broad spectrum of severity ranging from mild muscle hypotonia, manageable on an outpatient basis, to sudden death caused by respiratory arrest (3). The clinical course of infant botulism tends to be slowly progressive and is followed by a long recovery period. Return of autonomic function may be slower than neuromuscular function. The clinical picture can vary greatly depending on the severity of infection (15).

Clostridium botulinum is the main causal agent of this toxemia, but some rare neurotoxigenic strains of *Clostridium butyricum* and *Clostridium baratii* have been implicated in cases of infant botulism (1, 7, 8, 16, 26). Clostridia spores are present in soil, their main reservoir, and they can be transported in dust particles and dispersed by the wind, exposing people repeatedly to botulism spores. Environmental exposure has been identified as an important risk factor for infant botulism (13, 21, 22, 30), especially in arid regions (14, 24). Honey consumption has also been identified as a risk factor for infant botulism (5, 15, 18, 27) although it may account for at most 20% of the cases (29). Moreover, in Argentina, botulinum spores have been detected in medicinal plants commonly given to infants as household remedies (9, 10, 28).

A case of infant botulism is defined as laboratory-confirmed botulism occurring at 12 months of age or less (without ingestion of BoNT already present in food), where patients present a characteristic flaccid paralysis and BoNT in serum and/or where BoNT-producing clostridia are identified in a patient's feces or enema specimen. As of 2006, 26 countries had reported the occurrence of at least one case of infant botulism among their inhabitants, and the largest numbers of cases have been reported, in descending order, by the United States, Argentina, Australia, Canada, Italy, and Japan (19). Remarkably, most countries have not reported infant botulism cases yet. This limited reporting of infant botulism contrasts with the known global occurrence of *C. botulinum* spores in soils and dust, and it suggests that infant botulism could be underrecognized, underreported, or both (20). At present, infant botulism is the most common form of human botulism in Argen-

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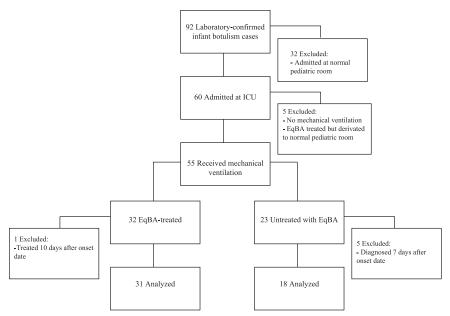∇ Published ahead of print on 14 September 2011.

FIG. 1. Enrollment, follow-up, and data analysis in the retrospective study. A total of 92 laboratory-confirmed cases of infant botulism diagnosed in Mendoza, Argentina (1993 to 2007), were evaluated for eligibility. Forty-nine patients were enrolled in the study; the rest of the patients were excluded because they were admitted to a normal pediatric room, did not receive mechanical ventilation, were diagnosed 7 days after onset the symptoms, and/or received EqBA 7 days after the onset of symptoms. ICU, intensive care unit.

tina (25) and in the United States (12). From 1982 to 2010, Argentina reported 605 laboratory-confirmed cases of infant botulism, with a mean of 39.5 cases per year in the last 10 years (M. I. Farace, Administración Nacional de Laboratorios e Institutos de Salud Dr. Carlos G. Malbrán, and R. A. Fernández, Área Microbiología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, unpublished data). The average annual incidence of infant botulism in Argentina is similar to that of the United States: 2.2 per 100,000 live births in Argentina (25) and 1.9 per 100,000 live births in the United States (12).

All cases of infant botulism registered in Argentina have been caused by BoNT type A, except one case caused by type B (9). In the United States, BoNT types A and B have been implicated in almost all cases of infant botulism (19).

Specific treatment of infant botulism consists in the administration of botulinum antitoxin. In the United States, the California Department of Public Health developed a human-derived botulism antitoxin (BabyBIG) exclusively for treatment of infant botulism (4, 6). BabyBIG is safe and effective for infant botulism type A and type B, and it was shown to be cost-effective by U.S. standards (23). However, its high cost ($45,300 per vial) (23) may not be affordable for people or health systems of many countries, including Argentina. Commercial equine botulinum antitoxin (EqBA) has been available in the United States since 1940 (4), but it has rarely been used in infant botulism cases because of the risk of inducing lifelong hypersensitivity to equine antigens, its short half-life (5 to 8 days), and lack of evidence of its benefit (12, 15). While up to 9% of patients might develop hypersensitivity to equine sera, severe reactions are rare (23). In this study, we report the effectiveness and safety of the EqBA as treatment for infant botulism.

MATERIALS AND METHODS

Study design. This is an analytical, observational, retrospective, and longitudinal study.

Patients and eligibility. We reviewed medical records of infant botulism cases that occurred in Mendoza, Argentina, from January 1993 to December 2007. Data regarding clinical features, laboratory diagnosis, and treatment were extracted from each medical record.

We considered the following inclusion criteria: (i) laboratory confirmation of botulism, (ii) requirement of intensive care and mechanical ventilation, and (iii) treatment with EqBA within 5 days from the onset of signs (for patients who received EqBA). This enrollment limitation was decided based on the concern that any efficacy of EqBA could decrease over time as motor-nerve intoxication continues (6).

Clinical diagnosis was confirmed by detection of BoNT in stool or serum. Identification of *C. botulinum* in stool samples supported the diagnosis. Fecal and enema samples were tested by established methods to identify BoNT and BoNT-producing clostridia (17, 25). Laboratory diagnosis was carried out in Área Microbiología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo.

Study groups. In this retrospective study, medical records were reviewed in order to classify patients into two groups: (i) EqBA-treated patients, comprised of infants who received treatment with EqBA, and (ii) untreated patients, comprised of infants who did not receive antitoxin treatment (Fig. 1). Supporting treatment at the intensive care unit was the same for both groups: all patients received tube or intravenous feeding, upper airway clearing, mechanical ventilation, physical therapy, and enemas for clearing out the bowel, as needed. Vital functions were monitored in all cases.

Outcome measures. We evaluated the following variables: age, length of hospital stay, length of stay in the intensive care unit, number of days on mechanical ventilation, and number of days on tube feeding. The primary safety of EqBA was evaluated by the occurrence of adverse effects, including possible allergic reactions. The primary efficacy was determined by length of the hospital stay. Secondary efficacy was evaluated by duration of stay in the intensive care unit, number of days on mechanical ventilation, and number of days on tube feeding. Additionally, we analyzed secondary complications: digestive, neurological, and cardiovascular infections (sepsis); pneumonia (associated with mechanical ventilation or caused by aspiration); immunological complications; and sequelae.

Description of EqBA and its administration. The following was extracted from the clinical records. Specific treatment was conditioned by the availability of EqBA, which was supplied by the Public Department of Health. Each

TABLE 1. Desensitization to EqBA by subcutaneous route

Dose no.[a]	Dilution in normal saline	Vol (ml)
1	1:20	0.05
2	1:10	0.10
3	1:10	0.30
4	Undiluted	0.10
5	Undiluted	0.20
6	Undiluted	0.50

[a] Each dose was administered consistently at 20-min intervals.

vial of EqBA contained 7,500 IU of type A and 5,500 IU of type B. The antitoxin was administered as a single intravenous slow infusion (4 to 6 h) of 500 IU (type A) per kilogram of body weight, diluted in 0.9% saline at a 1:10 dilution.

Before administration of EqBA, patients were subjected to a test of sensitivity, which consisted of an intradermal injection of 0.1 ml of EqBA at a dilution of 1:1,000 (in distilled water). Three patients presented sensitivity-positive test results and were subjected to desensitization. This procedure was carried out by serial subcutaneous injections of antitoxin at intervals of 20 min (Table 1). In all cases, the EqBA used was a bivalent botulism antitoxin (AB) produced by Aventis Pasteur. It is a licensed product supplied in single-dose vials and consists of a refined and concentrated liquid preparation of horse (equine) globulins modified by enzymatic digestion. Each vial contains the following: 7,500 IU (equivalent to 2,381 U.S. units) of type A and 5,500 IU (equivalent to 1,839 U.S. units) of type B.

Statistical analysis. Analysis of data was performed with standard statistical software (Statistix, version 7.0; Analytical Software, Tallahassee, FL). Differences between treated and untreated patients (quantitative continuous variables) were examined with a Mann-Whitney test with a significance level of 0.05. Proportions were analyzed by Fisher's exact test.

RESULTS

Between January 1993 and December 2007, 92 infant botulism cases were registered in Mendoza, Argentina. All of them were caused by *C. botulinum* type A. The lethality in this period was 5.4% (5/92). The laboratory diagnosis of the five patients who died was confirmed after a week of the onset of symptoms. According to the inclusion criteria, patients admitted to a normal pediatric room, patients that did not need mechanical ventilation, and patients treated with EqBA after 5 days from the onset of symptoms were excluded. Forty-nine out of the 92 laboratory-confirmed cases met the inclusion criteria. All patients received supportive treatment, which consisted of mechanical ventilation, tube or intravenous feeding, upper airway hygiene, physical therapy, monitoring of vital functions, and enema, if necessary. Thirty-one patients were treated with EqBA, and 18 were not treated with antitoxin (Fig. 1). The baseline characteristics of the two groups were similar. Laboratory diagnosis of the 49 patients was confirmed within 24 to 48 h after the arrival of the sample to the laboratory. The rapid laboratory diagnosis was essential because only patients with confirmed infant botulism received specific treatment with EqBA.

The mean length of the hospital stay of EqBA-treated infants was 23.9 days shorter than that in patients who did not receive EqBA ($P = 0.0007$) (Table 2). The secondary outcome measures were also significantly shorter in the EqBA-treated group: duration in intensive care was shortened by 11.2 days ($P = 0.0036$), duration on mechanical ventilation was shortened by 11.1 days ($P = 0.0155$), and duration on tube or intravenous feeding was shortened by 24.4 days ($P = 0.0001$) (Table 2). Regarding complications, the incidence of sepsis in EqBA-treated infants was 47.3% lower ($P = 0.0017$) than in untreated patients (Table 2). Severe residual hypotonia was noticed in four untreated infants while none of the EqBA-treated patients presented this sequela (Table 2). Hypotonic patients recovered by 90 days after discharge from the hospital. No adverse reactions attributable to administration of EqBA were noticed, except for one infant treated with EqBA who developed a transient erythematous skin rash.

In both groups, EqBA-treated and untreated patients, physicians followed the standardized criteria for hospital discharge: no further need for inpatient care, no need for mechanical ventilation for at least 3 days, no worsening of paralysis in the previous 3 days and a demonstrated improvement in motor and bulbar function, and 3 days of intake by

TABLE 2. Results of safety and primary and secondary efficacy in 49 patients with infant botulism

Parameter	Value for the group		P value
	Untreated	EqBA-treated	
No. of patients	18	31	
Age (mos.) at admission (mean ± SEM [range])	4.0 ± 0.5 (1–7)	3.5 ± 0.3 (1–7)	NS[b]
Outcome variable (mean ± SEM [range])			
Length of hospital stay (days)	52.6 ± 6.8 (19–130)	28.7 ± 2.1 (12–60)	0.0007
Length of ICU stay (days)[a]	28.3 ± 4.3 (8–89)	17.1 ± 0.9 (8–30)	0.0036
Duration of mechanical ventilation (days)	25.4 ± 4.5 (7–88)	14.3 ± 0.9 (7–29)	0.0155
Duration of tube or intravenous feeding (days)	49.2 ± 6.1 (19–120)	24.8 ± 1.8 (12–50)	0.0001
Incidence of complication (no. of positive patients/total no. of patients [%])			
Bacteremia (sepsis)	12/18 (66.7%)	6/31 (19.4%)	0.0017
Pneumonia at admission	14/18 (77.8%)	20/31 (64.5%)	NS
Pneumonia during mechanical ventilation	18/18 (100%)	19/31 (61.3%)	0.0018
Incidence of sequelae (no. of positive patients/total no. of patients [%])			
Severe hypotonia	4/18 (22.2%)	0/31 (0%)	0.0144
Laryngeal stenosis	1/18 (5.6%)	0/31 (0%)	NS

[a] ICU, intensive care unit.
[b] NS, not significant.

tube feeding of 25% or less of maintenance volume and calories, with the remainder consumed by mouth.

DISCUSSION

Infant botulism is a severe neurological disease that frequently requires hospitalization in intensive care units and mechanical ventilation. Infant botulism is considered an orphan disease, and there was no adequate specific treatment available until the approval of BabyBIG by the Food and Drugs Administration (FDA), in October 2003 (4, 6, 11). Arnon and colleagues demonstrated that treatment of infant botulism with BabyBIG is safe and effective, reducing the severity and duration of the illness as well as the hospital cost (6). However, many countries, including Argentina, cannot afford the cost of the BabyBIG. For these reasons, the use of EqBA could be an alternative specific treatment for infant botulism when BabyBIG is not available.

Early administration of EqBA to adult patients with food-borne and wound botulism was associated with improved outcomes in retrospective and observational studies. Approximately 6% of adults with food-borne botulism had anaphylaxis or serum sickness when treated with one or two vials of EqBA (6). In the United States, few patients with infant botulism have been treated with EqBA because of its potential for lifelong sensitization to equine proteins and the possibility that anaphylactic reactions to the EqBA might be more severe in infants. However, the efficacy of EqBA for infant botulism treatment has never been evaluated in a controlled trial (12). Recently, in the United States, a patient with infant botulism caused by *C. baratii*, which produces neurotoxin type F, was treated with heptavalent EqBA (2). No adverse effect to the EqBA was reported in this patient.

In this study, we observed that prompt treatment with EqBA within 5 days from the onset of symptoms decreased the severity of the illness and the mean hospital stay. Infants that received EqBA showed a significantly shorter stay in intensive care, on mechanical ventilation, and on tube or intravenous feeding than patients who did not receive this specific treatment. Moreover, treatment with EqBA showed no serious adverse effects, and only one patient presented a transient, blush-like erythematous rash. However, it is important to note that a few patients treated with BabyBIG experienced a transient, blush-like erythematous rash, perhaps related to antitoxin (6).

Specific treatment of infant botulism should be initiated as soon as possible. For the cases reported here, early clinical suspicion and confirmation by the laboratory made possible the specific treatment within 5 days of the beginning of the symptoms. In the 49 cases of infant botulism analyzed, the laboratory diagnosis was obtained within 24 to 48 h after the arrival of the samples. This was possible because our laboratory is near the hospitals and is available every day of the year.

Even though the number of cases reported in this study is small, the results suggest that EqBA deserves careful consideration as an alternative specific treatment for infant botulism when BabyBIG is not available. A definitive statement on the role of EqBA in the treatment of infant botulism should be reached by a prospective double-blinded randomized trial comparing EqBA with placebo or even BabyBIG. A despeci-

ated equine immune globulin with lower antigenicity also deserves serious consideration.

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